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BIOLOGY AND CONTROL OF BOTRYTIS BLIGHT OF LENTIL AND ALFALFA

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BIOLOGY AND CONTROL OF BOTRYTIS BLIGHT OF LENTIL AND ALFALFA

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Table of Contents

Abstract 1

Summaries by Year 1

Technical Report

Alfalfa 3

Disease survey for blossom blight 3

Quicker diagnosis of blossom blight 7

Disease management with fungicides 10

Cultivar reaction 15

Temperature and rainfall 20

Lentil 22

Disease survey 22

Disease management with fungicides 24

Stand density 27

Cultivar reaction 30

Growth stage 33

Publications and Presentations 35

Other 38

Abstract

This 4-yr study, initiated in 1995, was supported by the Agriculture Development Fund, the Canadian Seed Growers Association and the Matching Investment Initiative of AAFC. It assessed the distribution and importance of diseases caused by *Botrytis cinerea* on alfalfa and lentil. On alfalfa, blossom blight occurred in western Canada each year. Techniques for diagnosis of the disease were investigated and passed on to producers. Application of Benlate (benomyl, DuPont) provided consistent and cost-effective control of blossom blight, with yield increases generally from 25-100%. A Minor Use registration for Benlate was supported with this data. Cultivar reaction to blossom blight and optimum conditions for flower infection were studied in the laboratory. The extension component of this study has been very successful as evidenced by invited presentations to all of the alfalfa seed producer associations in western Canada. On lentil, botrytis blight caused little or no economic damage in field crops during this study, even under irrigation. Fungicides reduced foliar infection, but disease levels were never high enough to evaluate yield response adequately. Similar problems were encountered in field studies of cultivar reaction and stand density. In each of these studies, only 1 of 7 station years provided strong data. Both studies indicate that cv. Eston is less susceptible to seed infection than Laird, probably due to its more upright growth habit and quicker maturity. CDC Gold was highly susceptible to seed infection. Botrytis blight on lentil is very strongly driven by weather. It requires cool, wet conditions for epidemic outbreak, and so it has almost disappeared from lentil crops in the recent dry cycle. However, is likely to reappear and cause substantial losses in the next wet cycle.

Summaries by Year

1995 - Surveys to assess the incidence and distribution of blossom blight of alfalfa were conducted across Saskatchewan in July and August. The incidence of *Botrytis cinerea* in flowers was generally high (> 50%) in July, but levels dropped substantially in August. Three fungicide trials were conducted in alfalfa seed production fields. There were significant differences in blossom blight incidence and alfalfa seed yield in response to fungicide at the only site where blossom blight levels were severe. On lentil, the incidence and distribution of stem and pod blight was also assessed in surveys in July and early August. The incidence of *B. cinerea* and *Sclerotinia sclerotiorum* in flowers was low in the southern and western regions of the province, but *B. cinerea* was isolated from over 50% of lentil flowers at several sites in the central grainbelt. One fungicide trial and one trial to assess the impact of stand density on stem and pod blight were conducted on lentil at Saskatoon.

1996 - In alfalfa, the incidence of blossom blight pathogens in/on flowers changed quickly in individual fields in response to changes in weather conditions. Three fungicide trials were conducted in commercial alfalfa seed production fields in Saskatchewan. Yield increases in the best treatments ranged from 25% to over 100% in these trials. In lentil, surveys to assess the incidence and distribution of stem and pod blight were carried out in July and August. The incidence of *B. cinerea* and *S. sclerotiorum* in flowers was generally low in southern Saskatchewan, but *B. cinerea* was isolated from over 50% of lentil flowers at several sites in the central grainbelt. Yield increases were too small to be economic in fungicide trials at three sites.

1997 - Blossom blight incidence in alfalfa was very low due to hot, dry conditions during flowering. An improved agar-based media for use in assessing fields for blossom blight was developed. Over years, foliar application of Benlate fungicide provided the most consistent control of blossom blight; Bravo occasionally produced substantial yield increases, but the results were not as consistent. Foliar application of the fungicide Dithane was tested for the first time; results were variable, but some treatments produced yield increases. In lentil, no botrytis blight developed in any trial, including those that were inoculated and irrigated.

1998 - Blossom blight levels were generally very low, due to hot dry conditions during flowering. Benlate continued to provide more consistent blossom blight control than the other fungicides in the tests. Small but consistent differences in disease reaction to *B. cinerea* and *S. sclerotiorum* among alfalfa cultivars were noted in greenhouse trials, but these differences need to be evaluated in the field before any recommendations to growers are warranted. Controlled environment studies demonstrated that *B. cinerea* is severely inhibited by hot weather.

General

1995 - The contract with ADF was signed in March of 1995 for $83,500 over 3 years. The CSGA contributed an additional $5000 per year and AAFC's Matching Investment Initiative also contributed $7000 per year. A summer student and short-term technicians were hired to assist with the field work, which was supervised and run by AAFC staff. Fungicides were donated by Rhone-Poulenc (Rovral), ISK Biosciences (Bravo) and Alberta Agriculture and Food (Benlate).

1996 - A cool, wet spring delayed seeding, and harvest was delayed until late into October. A graduate student from China, Mr. Z. Lan, joined the project in early January. Two summer students and a technical assistant for late fall work were hired. Fungicides were donated by BASF (Ronilan), ISK Biosciences (Bravo) and Alberta Agriculture and Food (Benlate).

1997 - Hot, dry conditions in summer and fall restricted disease development. Disease survey and fungicide trials on lentil were not undertaken due to the unfavourable conditions. As well, shortages of growth cabinet space caused by the demolition and rebuilding of the AAFC phytotron at Saskatoon delayed the laboratory studies. A summer student and a part-time technical assistant were employed on the project. Fungicides were donated by Rohm and Haas (Dithane), ISK Biosciences (Bravo) and Alberta Agriculture and Food (Benlate). A one year extension of the study was approved by ADF and CSGA.

1998 - As in 1997, hot, dry conditions in 1998 restricted grey mold development in most areas of the Canadian prairies. No fungicide trials in commercial fields or disease surveys on lentil were undertaken, but trials were conducted under irrigation at Saskatoon. Most of the work on blossom blight of alfalfa focused on laboratory studies, because hot dry weather limited disease potential in the field. Mr. Z. Lan completed the research for his M.Sc. project and started writing his thesis.

Alfalfa

Disease survey for blossom blight

Objective: Initially, to determine the extent of the area affected by blossom blight, and subsequently to obtain information on disease development, its relation to weather conditions, and to assist growers in making decisions regarding fungicide application.

1995 - The incidence of *B. cinerea* and *S. sclerotiorum* was assessed in 11 fields representing the production areas in Saskatchewan. Most sites were assessed in July and again in August. At each site, cooperators took 10 samples (4 blossoms per plate, 2 plates per site, 5 sites per field) at regular intervals along a teardrop-shaped transect of the field. For each sample, the oldest blossom from each of five racemes was picked with sterile forceps and plated, without surface sterilization, onto acidified PDA. The plates were then sent to Saskatoon for assessment. The incidence of pathogens in the florets was assessed about 10 days after sampling. The incidence of *B. cinerea* infestation was high (> 50%) in almost all of the fields in July (Table 1). By August, the incidence of flower infestation had fallen at most sites due to dry weather in July.

In spring and early summer, the central grainbelt had above-normal rainfall but northern regions were relatively dry. This was reflected in the level of leaf spot severity; the mean foliar disease severity in July was less than 35% in the north, but over 70% in the central grainbelt.

1996 - The incidence of *B. cinerea* and *S. sclerotiorum* was assessed in a survey of 23 fields in the major production areas of Saskatchewan. Sites were assessed two to five times, starting in July and continuing at 7 - 10 day intervals into August. In each field, the producer took samples as described above and sent the plates to Saskatoon for assessment. The results were highly variable and showed no clear pattern among sampling times at each site (data not shown). Pathogen incidence was generally low at early bloom. Mean incidence increased over time and by late bloom, incidence was moderate to high in most regions (Table 2). Both pathogens occurred at high levels in individual fields, but *B. cinerea* was generally the dominant pathogen when mean incidence was high. The two pathogens rarely occurred at high levels in the same field. This pattern has important implications for disease management, since many fungicides are not effective against both pathogens.

1997 - Samples were collected and assessed from 10 seed fields at weekly intervals for 4 to 5 weeks, starting at 50% bloom. Flower infestation was low (Table 3) due to hot, dry weather at flowering. Blossom blight pathogens were present at very low levels at early to mid-bloom, and only one field had moderately high levels at late bloom. Low levels of flower infestation (20-30%) can cause yield losses, but fungicide application is most cost-effective when infestation levels are high (>70%). Also, our working hypothesis is that high levels of flower infestation late in the bloom period are less important to yield than similar levels at early bloom. Fungicide applications would not have been economical on these crops, but there were a few localized areas where timely application of fungicide saved the crop.

Table 1. The incidence of *Botrytis cinerea* and *Sclerotinia sclerotiorum* on alfalfa flowers, and leaf spot severity and the dominant foliar pathogens at sites in Saskatchewan in 1995.

Location	Date	Botrytis (%)	Sclerotinia (%)	Leaf spot severity (%)
Northwest region				
- Shellbrook 1	July 17	68	nd	19 - YLB, CLS, BS
	Aug. 1	nd	nd	58 - CLS, BS, YLB
- Shellbrook 2	July 17	60	nd	12 - YLB, CLS, BS
	Aug. 1	20	nd	41 - YLB, BS, CLS
- Shellbrook 3	July 17	63	nd	70 - YLB, CLS, BS
	Aug. 1	48	nd	74 - BS, YLB, CLS
- **Mean**	**July**	**64**	**nd**	**34%**
Northcentral				
- Tisdale	July 11	90	3	34 - BS, CLS
	Aug. 4	60	25	74 - CLS, BS
- Ridgedale	July 18	78	nd	37 - BS, CLS
	Aug. 4	10	0	43 - BS, YLB, CLS
- Melfort	Aug. 8	53	3	20 - BS, CLS
- Arborfield	July 11	60	0	22 - YLB, BS
	Aug. 3	23	nd	50 - BS, CLS, YLB
- MacDowall	July 19	3	0	nd
	Aug. 1	12	5	nd
- **Mean**	**July**	**58**	**1**	**31%**
Northeast				
- Tobin Lake 1	July 12	65	0	15 - BS, CLS, YLB
	Aug. 3	53	nd	30 - CLS, BS, YLB
- Tobin Lake 2	July 12	65	0	15 - YLB
	Aug. 3	15	nd	23 - BS, YLB
- **Mean**	**July**	**65**	**0**	**15%**
Central				
- Watson	July 10	73	8	nd
	July 27	93	5	63 - BS, YLB, CLS
	Aug. 15	14	6	29 - BS, CLS, YLB
- Pilger	July 27	48	3	81 - BS, CLS
	Aug. 15	7	6	34 - CLS, BS
- **Mean**	**July**	**71**	**15**	**72%**

BS = black stem (*Phoma medicaginis*), CLS = common leaf spot (*Pseudopeziza medicaginis*), YLB = yellow leaf blotch (*Leptotrochila medicaginis*). The leaf spot diseases are listed in descending order of importance for each site. nd = not done

Table 2. Incidence (mean % and *range*) of *Botrytis cinerea* (Bc) and *Sclerotinia sclerotiorum* (Ss) on alfalfa flowers in commercial fields in Saskatchewan and Manitoba, 1996.

Location	No. of fields	Early bloom		Mid bloom		Late bloom	
		Bc	Ss	Bc	Ss	Bc	Ss
SASKATCHEWAN							
Northwest	3	25	23	24	33	30	12
		7-44	*8-36*	*0-40*	*5-69*	*8-51*	*5-18*
Northcentral	4	15	18	41	35	54	15
		4-31	*1-33*	*15-93*	*10-58*	*18-100*	*9-21*
Northeast	9	8	13	8	17	58	28
		3-20	*0-38*	*0-33*	*0-58*	*20-99*	*3-63*
Central	3	11	26	9	8	7	8
		0-19	*0-67*	*0-26*	*0-13*	*7*	*8*
Southeast	4	6	21	14	28	67	28
		3-10	*4-53*	*10-20*	*8-59*	*31-93*	*7-63*
MEAN	**23**	**12**	**18**	**19**	**25**	**53**	**22**
MANITOBA							
Eastern	2	5	0	nd	nd	58	23
		5	*0*	nd	nd	*15-100*	*5-40*
Southern	2	13	9	nd	nd	69	54
		5-20	*5-13*	nd	nd	*38-100*	*30-78*
Interlake	2	2	4	nd	nd	35	44
		0-3	*0-8*	nd	nd	*18-52*	*33-55*
MEAN	**6**	**7**	**4**	**nd**	**nd**	**54**	**40**

nd = not done.

Table 3. Infestation of flowers (%) with *Botrytis cinerea* and *Sclerotinia sclerotiorum* in 10 fields in Saskatchewan in 1997.

Region	No. of fields	Bloom stage	% Infestation by *B. cinerea*	% Infestation by *S. sclerotiorum*
South	3	Early	0 - 10	0
		Middle	0	0 - 5
		Late	0	0
Central	2	Early	0 - 10	13 - 18
		Middle	3 - 5	3 - 18
		Late	0 - 3	0 - 18
North	5	Early	0 - 5	0 - 3
		Middle	0	0
		Late	0 - 58	0 - 38
Mean			**4.4**	**5.1**

1998 - Twenty-five commercial alfalfa seed fields in Saskatchewan and Manitoba were surveyed at 7-10 day intervals using a test kit with a (new) semi-selective media. With this improved media, the percentage of infested flowers could be assessed 5-6 days after sampling, instead of 10 days. Growers kept one set of samples to rate, based on pictures in a booklet that was part of the test kit, and sent a second set of samples to Saskatoon for comparison. In Saskatchewan, most of the samples were collected by growers, and in Manitoba, most of the sampling was done by research staff or seed companies.

Weather conditions during flowering were hot and dry across most of Saskatchewan in 1998. Consequently, the incidence of both pathogens was low. The exception was an area in northeastern Saskatchewan that received heavy rainfall at mid and late flowering. At late bloom, 93% of flowers in one field were infested with *B. cinerea* and disease was severe.

Cool, wet conditions were prevalent in Manitoba at early flowering, and the pathogens occurred at moderate to high levels in most areas (Table 4). As a result, many fields were treated with fungicide to protect flowers from infection. The incidence of both pathogens declined dramatically with the onset of hot, dry weather at most sites at late bloom.

Conclusion - Surveys over four years have demonstrated that blossom blight is present at sites across the prairie region each year. There is a strong association between prolonged cool, wet conditions and blossom blight outbreaks. Alfalfa seed producers on the prairies recognize this disease, which was only identified here in 1993, as an important constraint to production and have supported this work to identify and manage the disease. There are several factors that reduce alfalfa seed yield under wet conditions; flowering is reduced, pollinators are less active and foliar disease severity increases. Blossom blight is only a part of this complex of factors.

Table 4. Flower infestation (% and range) by *Botrytis cinerea* (*Bc*) and *Sclerotinia sclerotiorum* (*Ss*) in commercial alfalfa seed fields in Manitoba and Saskatchewan, 1998.

Location and no. of fields	Early bloom		Mid-bloom		Late bloom	
	Bc	*Ss*	*Bc*	*Ss*	*Bc*	*Ss*
Saskatchewan (17 fields)	1 (0-3)	8 (0-15)	8 (0-30)	10 (0-35)	17 (0-93)	1 (0-4)
Manitoba (8 fields)	13 (0-25)	23 (0-50)	21 (0-50)	22 (0-50)	1 (0-31)	5 (0-38)

Quicker diagnosis of blossom blight

Objective - To develop techniques that would permit more rapid diagnosis of moderately severe levels of blossom blight.

When blossom blight is severe, it is easy to identify because many of the racemes are covered with a grey mold or contain the sclerotia bodies of *S. sclerotiorum*. However, at that point, it is generally too late to save the crop. When the disease is increasing but not yet severe, the symptoms are quite subtle; the oldest flowers abscise prematurely, but there are no distinct lesions on the flowers that can be used as a cue for disease identification. The presence of fungal mycelium in the oldest flowers of the raceme provides a good diagnostic characteristic. In healthy racemes, the oldest flower will pull away cleanly from the peduncle. In infected racemes, other florets often come away as well, held together by threads of mycelium. We demonstrated this technique to growers many times, but most growers wanted something more definite to determine if a fungicide application was warranted. Therefore, we initiated studies to develop a grower test kit to make disease identification quicker.

1997 - Sporulation of *B. cinerea* and formation of sclerotia of S. *sclerotiorum* were found to occur very quickly on potato dextrose agar (PDA, a basic agar media) amended with chopped alfalfa leaves and kept in the dark for the first three days. Antimicrobial compounds were assessed to determine if they reduced contamination and made assessment of the media plates in the test kit quicker or more reliable. For most treatments, 50 g of fresh, homogenized alfalfa leaves (L) were added per litre of media. The antimicrobial amendments were: 5 mg/l pimaricin (P), 50 mg/l novobiocin (N), 200 mg/l chloramphenicol (C), and 3 ml (85%) lactic acid/l (aPDA). The treatments were PL, NL, CL, PNL, PCL, aPDA, aPDAL, and PDA with no amendments. N, P and lactic acid are heat stable. In the first two tests, one treatment (aPDAL) was not included. aPDA was the media used in the blossom blight survey in 1995-96.

At five sites (replications) in a field, the oldest floret from each of 8 racemes was plated onto the treatment media, with 4 florets per plate. The plates were incubated in the dark at room temperature for three days (Day 3), then moved to 12 hr day/night cycles. The incidence of bacterial and fungal contaminants was assessed at Day 3, and the incidence of flowers with *B. cinerea* or S. *sclerotiorum* was assessed daily from Day 5 to (at least) Day 10. The experiment

was repeated six times, twice in a commercial alfalfa field at Watson, and four times at the AAFC Research Farm in Saskatoon. At Saskatoon, mist irrigation was applied for a short period each evening in Tests 4 to 6. The plants were inoculated with *B. cinerea* prior to Test 4 and Test 5, and with *S. sclerotiorum* prior to Test 6.

In Test 1 at Watson, the incidence of *B. cinerea* was very low (mean < 5%, not shown). For *S. sclerotiorum*, the highest incidence was found on aPDA (Table 5). In the second sampling at Watson, the final incidence of *B. cinerea* was 73% on PCL and 70% on aPDA, both of which were much higher than on the other media. The incidence on *S. sclerotiorum* was 35% on PCL and 25% on aPDA. Less than 20% incidence was observed on the other media. There was a similar trend in Tests 3 and 4 on the AAFC Research Farm at Saskatoon. The highest incidence of *B. cinerea* and S. *sclerotiorum* occurred on aPDA, but levels were low (data not shown).

In Tests 5 and 6, the incidence of both pathogens on aPDAL approached their maximum values on Day 5 (Table 6). Sporulation (and therefore, identification) generally occurred more quickly on aPDAL than on aPDA. The incidence of *S. sclerotiorum* was low, but was higher on aPDAL than on the other media at Day 5 in both tests (Table 6).

There were large differences among the media in contamination at Day 3. Chloramphenicol (an antibiotic) consistently produced better results than novobiocin (Table 7). Pimaricin (a fungicide) was ineffective by itself, but provided the best control in the trial when used in combination with chloramphenicol. However, lactic acid provided excellent control of both fungi and bacteria (Table 7) and is much cheaper and easier to use than pimaricin or the antibiotics.

Table 5. Impact of growth media on incidence (%) of *Botrytis cinerea* and *Sclerotinia sclerotiorum* on alfalfa flowers collected on two dates (tests) at Watson, SK (n = 5).

	Test 1		Test 2	
	S. sclerotiorum		*B. cinerea*	*S. sclerotiorum*
Media	Day 6	Day 10	Day 14	Day 14
PDA	20	55	13	3
PL	15	60	5	8
NL	8	58	20	18
CL	13	55	23	10
PNL	5	38	18	13
PCL	10	63	73	35
aPDA	28	93	70	25

PDA = potato dextrose agar, L = alfalfa leaves, P = pimaricin, C = chloramphenicol, N = novobiocin, a = lactic acid.

Table 6. Incidence (%) of *Botrytis* and *Sclerotinia* on alfalfa flowers on different media at Saskatoon in 1997 (n = 5).

Media	Test 5		Test 6	
	Day 5	Day 10	Day 5	Day 10
Botrytis cinerea				
PDA	10 d	70 abc	3 c	35 d
PL	13 d	58 bc	10 c	45 bcd
NL	23 cd	53 c	13 c	43 cd
CL	33 c	63 bc	8 c	50 bcd
PNL	33 c	58 bc	3 c	40 cd
PCL	55 b	78 ab	33 b	73 ab
aPDA	8 d	83 ab	3 c	68 ab
aPDAL	90 a	93 a	60 a	90 a
Sclerotinia sclerotiorum				
PDA	0 b	13 ab	5 b	8 c
PL	0 b	8 b	3 b	15 bc
NL	0 b	8 b	10 b	20 abc
CL	0 b	8 b	13 b	20 abc
PNL	0 b	10 b	0 b	15 bc
PCL	0 b	20 ab	10 b	35 ab
aPDA	3 b	28 a	10 b	23 abc
aPDA L	10 a	23 ab	28 a	38 a

Means in columns followed by the same letter did not differ based on Duncan's Multiple Range Test at $P \leq 0.05$.
PDA = potato dextrose agar, L = alfalfa leaves, P = pimaricin, C = chloramphenicol, N = novobiocin, a = lactic acid.

aPDAL induced early sporulation of both pathogens and provided effective reduction in contamination. The base media (PDA) is commercially available and lactic acid is inexpensive, heat stable, and does not require separate sterilization. Therefore, aPDAL would be an effective and useful medium for use in a test kit for producers.

Conclusion - This preliminary work was very promising, but the resources and time required to pursue further development were not available within the scope of the project. Therefore, funding from AFIF was obtained for 1998 and a test kit for blossom blight assessment was developed. A number of selected growers used the kit and compared their results with samples sent to our laboratory for confirmation. Comments from these producers were generally positive, so the test kit and manual will be revised this winter and tested again in 1999.

Table 7. Colonies of contaminants (% of the maximum level) at Day 3 on alfalfa flowers incubated on various media (n = 5).

Media	Test 1	Test 2	Test 5	Test 6	Mean
PDA	100 b	87 cd	100 d	100 c	97
PL	62 ab	100 d	96 d	73 bc	83
NL	35 a	68 cd	62 bcd	44 b	52
CL	24 a	48 bc	43 abc	38 ab	38
PNL	52 ab	70 cd	68 cd	37 ab	57
PCL	7 a	19 a	6 a	21 a	13
aPDA	30 a	35 ab	25 ab	21 a	28
aPDAL	-	-	17 a	41 ab	29
Maximum level	**47**	**107**	**61**	**52**	

Means in columns followed by the same letter did not differ based on DMRT at $P \leq 0.05$.
PDA = potato dextrose agar, L = alfalfa leaves, P = pimaricin, C = chloramphenicol, N = novobiocin, a = lactic acid

Disease management using fungicides

Objective: To identify fungicides that provided cost-effective control of blossom blight and are readily available to commercial alfalfa producers in western Canada.

1995 - Trials to assess the efficacy of three fungicides (Benlate at 0.8 kg a.i. ha^{-1}, Bravo at 1.4 kg a.i. ha^{-1}, and Rovral at 1.1 kg a.i. ha^{-1}) for management of blossom blight were established in growers' fields at three sites in Saskatchewan. The treatments were one vs two applications of each fungicide, and an untreated control. Each plot was 12 x 12m. There were two replications per site in a randomized complete block design at MacDowall (cv. Rambler) and Pilger (cv. Beaver) and four replicates at Watson (cv. Algonquin). The fungicides were applied at 45 psi and high water volumes using a truck-mounted sprayer with a 7.6 m spray boom. The treatments were applied on 10 and 19 July at MacDowall, 11 and 17 July at Pilger, and 7 and 17 July at Watson. Samples (the oldest flower from each of 20 racemes) were collected from each plot on 19 July and 01 August at MacDowall, 27 July at Pilger, 10 and 27 July at Watson, and plated onto aPDA. The number of flowers infested with *B. cinerea* and *S. sclerotiorum* were assessed 10 days after collection. At MacDowall and Pilger, two $1m^2$ samples were harvested per plot. At Watson, two 12 x 1.6m strips were direct-combined in each plot. In Manitoba, collaborators conducted strip-plot spray trials; flower samples were sent to Saskatoon for assessment.

The incidence of *S. sclerotiorum* was low at all sites. The incidence of *B. cinerea* was low in Manitoba and at MacDowall in Saskatchewan, but was high at Pilger and Watson. At Watson, a

small sample of flowers collected on 01 July was 100% infested with *B. cinerea* and blossom blight caused substantial flower abortion before the fungicide treatments were applied.

In Saskatchewan, the only difference ($P \leq 0.05$) in pathogen incidence was observed after the early spray date at Watson (Table 8). All of the fungicides provided some control of *B. cinerea* at that time, but Benlate was most effective. Florets collected near the end of the flowering period were heavily infected with *B. cinerea*. Watson was also the only site where fungicide application increased seed yield. Both Benlate and Bravo increased ($P \leq 0.05$) yield relative to the control. A similar trend was noted at Pilger, but the differences were not significant. Fungicide application did not affect pathogen incidence at the sites in Manitoba. Rovral had no effect at any site.

Table 8. The impact of three foliar fungicides on the incidence (%) of *Botrytis cinerea* and *Sclerotinia sclerotiorum* (in brackets) on alfalfa flowers and seed yield (kg/ha) at sites in Saskatchewan and Manitoba in 1995.

Location & Date	Benlate		Bravo		Rovral		
	1 appl	2 appl	1 appl.	2 appl.	1 appl.	2 appl.	Control
SASKATCHEWAN							
- MacDowall							
July 19	0 (0)	10 (5)	3 (0)	23 (0)	5 (0)	3 (0)	3 (0)
August 1	13 (0)	20 (0)	13 (3)	38 (0)	23 (5)	23 (5)	13 (5)
Seed yield (kg/ha)	**300**	**175**	**230**	**175**	**245**	**175**	**250**
- Pilger							
July 27	60 (3)	58 (10)	58 (3)	88 (0)	70 (5)	75 (0)	48 (3)
Seed yield (kg/ha)	**185**	**150**	**175**	**190**	**120**	**110**	**100**
- Watson							
July 10	11 (13)	6 (3)	53 (10)	64 (6)	50 (13)	55 (16)	73 (8)
July 27	96 (4)	89 (3)	94 (4)	98 (4)	99 (5)	90 (1)	93 (5)
Seed yield (kg/ha)	**130**	**240**	**215**	**165**	**135**	**140**	**120**
MANITOBA							
- Miami	0.4 (0.4)	-	1.6 (15)	-	-	-	0.4 (6)
- Arborg	2.0 (0)	-	0.0 (0)	-	-	-	2 (0)
- Lac du Bonnet	18 (6)	-	14 (14)	-	-	-	22 (10)
- Seven Sisters Falls	6 (8)	-	6 (4)	-	-	-	24 (0)

Fungicides were applied either one or two times per plot in Saskatchewan, but once in Manitoba; Rovral was not included at sites in Manitoba.

1996 - Several minor modifications were made to the protocol: 1) Ronilan 50% (BASF) at 0.5 kg a.i./ha replaced Rovral (both Ronilan and Rovral are registered for control of *B. cinerea* and *S. sclerotiorum* on other crops and are used on canola for control of white mold in western Canada), 2) plot size was reduced to 6 x 12m to accommodate more treatments, 3) a late-spray-only treatment was added, and 4) all trials had 4 replications. Three tests were set out in growers' fields in Saskatchewan; cv. Rambler at MacDowall, cv. Orca at Parkside and cv. Vernal at

Atwater. Flowering was delayed by cool, wet conditions in late June and early July, so the treatments were not applied until July 24 and August 1 at MacDowall, July 17 and 26 at Parkside and July 18 and 29 at Atwater. Samples of blossoms, consisting of the oldest flower from each of 20 racemes, were collected from each plot on the day of the second fungicide application and plated onto aPDA. One additional set of samples was taken at Atwater on August 14. The plots were harvested (30 m^2 per plot) in late October. In Manitoba, cooperators compared a single application of Benlate with an untreated control in strip-plot trials with two replicates at six sites.

Early application of Benlate substantially reduced the incidence of *B. cinerea* at Parkside and MacDowall (Table 9). Yields were increased by 25% at Atwater, where the fungicides had only a small impact on pathogen incidence. Yield increases were larger (50-100%) at MacDowall and Spiritwood, where fungicide application had a greater impact on pathogen incidence (Table 10).

In Manitoba, the Benlate spray treatment reduced pathogen incidence in early August, but the effect on yield was not consistent (Table 11).

Table 9. Impact of a single fungicide application on the incidence (%) of *Botrytis cinerea* (Bc) and *Sclerotinia sclerotiorum* (Ss) in three alfalfa seed fields in Saskatchewan, 1996.

	Parkside		Atwater		MacDowall		Mean	
Fungicide	Bc	Ss	Bc	Ss	Bc	Ss	Bc	Ss
Benlate	53a	0a	85a	23a	10a	10a	49a	11a
Bravo	88b	10a	85a	23a	58b	0a	77b	11a
Ronilan	90b	0a	78a	15a	45b	0a	71b	5a
Control	98b	18a	80a	20a	48b	8a	75b	15a

Means in columns followed by the same letter did not differ based on DMRT at $P \leq 0.05$.

Table 10. Impact of fungicide application on seed yield (kg/ha) in three commercial alfalfa seed fields in Saskatchewan, 1996 (n = 4).

Fungicide	Parkside	Atwater	MacDowall
Benlate			
- early	150ab	400abcd	190a
- late	140abc	370 d	190a
- early + late	190a	470a	170ab
Bravo			
- early	110 bc	450abc	170ab
- late	130 bc	380 cd	160ab
- early + late	120 bc	470ab	110 b
Ronilan			
- early	110 bc	420abcd	140ab
- late	110 bc	360 d	140ab
- early + late	100 bc	390 bcd	130ab
Control	90 c	380 cd	130ab

Means in columns followed by the same letter did not differ based on DMRT at $P \leq 0.05$.

Table 11. Incidence (%) of *Botrytis cinerea* (*Bc*) and *Sclerotinia sclerotiorum* (*Ss*) in alfalfa flowers after one application of Benlate and seed yield (kg/ha) in six commercial seed fields in Manitoba, 1996 (n = 2).

	Benlate			Control		
Location	*Bc*	*Ss*	Yield	*Bc*	*Ss*	Yield
Seven Sisters Falls	58	23	240	100	5	220
Miami	20	13	280	78	35	360
Fisher Branch	18	20	180	53	33	170
Rosenort	33	63	100	38	78	120
Arborg	15	45	440	18	55	350
Lac du Bonnet	33	25	230	15	40	290
Mean	**30**	**32**	**248**	**50**	**41**	**252**

1997 - Tests were set out in growers' fields at MacDowall and Watson, SK. At MacDowall, Benlate and Bravo were applied at early and/or mid-bloom (6 treatments + a nonsprayed control) to assess the impact of fungicide timing. At Watson, moderate levels of *S. sclerotiorum* were found in one field at mid-late bloom, so a single application of fungicides (Benlate, Bravo, and Dithane (mancozeb) at 1.6 kg a.i. ha^{-1}) was made on July 17. Dithane was evaluated because it is inexpensive, is registered for *Botrytis* control in other crops, and has recently become readily available in western Canada because it is used in potato production.

There were no differences among treatments at MacDowall because disease levels were very low. At Watson, Benlate produced the highest yield (580 kg/ha), Bravo (560 kg/ha) and Dithane (550 kg/ha) were intermediate, and the untreated control (490 kg/ha) was lowest. The difference between Benlate and the control was significant.

In Manitoba, 2-3 applications of Dithane increased yield at some sites, but yields with Benlate were generally higher. Yields in Ronilan treatments were often lower than the untreated control (Smith and Huebner 1997, pers. comm.).

1998 - Trials were set out at MacDowall, Rosthern and Saskatoon, Saskatchewan, comparing a single application of Benlate, Bravo or Dithane with a nonsprayed control. Pathogen incidence was very low (Table 12) due to the hot, dry conditions that occurred across Saskatchewan, and the fungicides did not consistently reduce disease (Table 12) or increase yield (Table 13).

Table 12. Impact of fungicides applied at flowering on the incidence (%) of *Botrytis cinerea* (*Bc*) and *Sclerotinia sclerotiorum* (*Ss*) on alfalfa flowers at three sites in Saskatchewan, 1998 (n = 4).

	Rosthern		MacDowall		Saskatoon		Mean	
Fungicide	*Bc*	*Ss*	*Bc*	*Ss*	*Bc*	*Ss*	*Bc*	*Ss*
Control	10	1	8	4	8 b	0	8	2
Bravo	16	4	9	3	0 a	0	8	2
Dithane	9	4	5	8	9 b	1	8	4
Benlate	15	4	4	8	0 a	0	6	1
	NS	NS	NS	NS	*	NS	NS	NS

NS - Not significant. * Differences significant at $P \leq 0.05$. Means followed by the same letter do not differ.

Table 13. Impact of fungicides applied at flowering on alfalfa seed yield (kg/ha) at three sites in Saskatchewan, 1998 (n = 4).

Fungicide	Rosthern	MacDowall	Saskatoon	Mean
Control	38	312	149	166
Bravo	39	334	157	177
Dithane	49	366	144	186
Benlate	28	353	149	177
	NS	NS	NS	NS

NS - Not significant at $P \leq 0.05$.

Two fields where producers applied Benlate with commercial equipment and left untreated test strips were also assessed. At Atwater, the weather had been very wet early in the bloom period,

but turned hot and dry after the fungicide application, so the treatment did not improve yield. At Ridgedale, the weather was cool and wet after fungicide treatment; application reduced pathogen incidence and increased yield by 17% (although this difference was not significant, Table 14).

In addition, a trial was set out in two commercial fields to examine the impact of Benlate application at different points along a slope. The objective was to determine if it would be more cost-effective to treat only portions of the field, e.g. hilltops vs. low spots. Pathogen incidence was too low to show any effect.

Table 14. Impact of commercial-scale fungicide application on incidence (%) of *Botrytis cinerea* (*Bc*) and *Sclerotinia sclerotiorum* (*Ss*), and alfalfa seed yield (kg/ha) in two sites in 1998.

	Atwater			Ridgedale			Mean
Fungicide	*Bc*	*Ss*	Yield	*Bc*	*Ss*	Yield	Yield
Benlate	1	3	540	0	0	440	490
Control	4	1	530	59	0	370	450
	NS	NS	NS	*	NS	NS	

NS - not significant. * Difference significant at $P \leq 0.05$.

Conclusion - A large number of fungicide trials have been conducted in Saskatchewan and parallel trials have been run with cooperators in Manitoba and Alberta. In 1994, a temporary registration of Benlate was granted for blossom blight management in alfalfa. Our results in 1995 were used to support it for Minor Use registration. Benlate has provided the most consistent control of blossom blight across all of the sites in this trial. At sites where blossom blight was severe, yield increases have ranged from 25-100%. In Manitoba, growers and extension specialists have reported yield increases of 25% with Benlate application in the absence of disease, but our data from Saskatchewan does not support this observation. Bravo has also produced substantial yield increases at some sites, but the increases have not been as consistent as Benlate. Producers are interested in Dithane because it is much less expensive to apply than Benlate. Manitoba Agriculture applied for a Minor Use registration in 1998 for Dithane on alfalfa to manage blossom blight, but our data does not support registration at this time. In both 1997 and 1998, conditions were hot and dry during flowering in Saskatchewan and southern Alberta, and relatively few acres were sprayed with fungicide. Conditions in Manitoba were cool and wet over this period and a large proportion of the alfalfa seed production acreage was sprayed with fungicide to protect the crop.

Cultivar reaction

Objective: To determine if there are differences among alfalfa cultivars in susceptibility to *Botrytis cinerea*.

1995 - Microplots of 12 alfalfa cultivars were established from transplants at the Research Farm at Saskatoon. These cultivars were selected to represent a range of winter hardiness and plant

architecture types. There were seven cultivars with *Medicago sativa* spp. *sativa* backgrounds (OAC Minto, Iroquois, Apollo II, DK 135, Apica, Oneida-VR, Saranac) and five with high proportions of *M. sativa* spp. *falcata* (AC Nordica, Heinrichs, Vernal, Algonquin, and Beaver). Each plot was $1m^2$, and consisted of 16 seedlings at 0.25 m spacings. The plots were arranged in a randomized complete block design with five replicates. Also, 40 plants per cultivar were established in the greenhouse for assessment of disease reaction under controlled conditions.

1996 - The microplots were irrigated and inoculated to run-off with a spore suspension (1×10^5 spores/ml) of *B. cinerea*. Hot, dry conditions occurred after the inoculation treatment and little disease developed. A severe epidemic of verticillium wilt developed in the greenhouse-grown plants, so they were destroyed to prevent movement of the disease into valuable germplasm collections and new plants were started.

1997 - Plants were grown to flowering in pots in a greenhouse. Isolates of *B. cinerea* from alfalfa were maintained on PDA. Spores from 2-week-old cultures (1×10^5 spores/ml) were sprayed to run-off onto alfalfa flowers and the plants were sealed in a clear plastic bag for 48 hours. Plants were incubated at 20°C and 12 hour day. In these preliminary experiments, samples were taken at intervals ranging from 12 to 48 hours, with 25 flowers per cultivar in each of four replicates. These flowers were surface sterilized, plated onto PDA and the incidence of *B. cinerea* was assessed 10 days after collection.

The disease reaction to *B. cinerea* differed among alfalfa cultivars in these preliminary results, but the differences were not consistent among sampling times. A new set of trials, using detached flowers to improve control of conditions for infection and incubation, was initiated.

1998 - Tests of cultivar reaction were conducted on detached flowers (Fig. 1), on whole plants in the greenhouse (Fig. 2) and on mist-irrigated microplots in the field (Fig. 3). None of the cultivars were resistant, but there were consistent differences in the degree of susceptibility: cvs. DK 135, OAC Minto, and Iroquois were significantly less susceptible than cvs. Algonquin, Apollo II and Heinrichs. In microplots in the field, the incidence of flower infection three days after inoculation was 3-6% for the less susceptible group compared to 20-22% for the susceptible group. In whole plant inoculation tests under controlled conditions, the incidence of flower infection was 36-40% in the moderately susceptible group and 71-79% in the highly susceptible cultivars. The detached flower assays showed similar differences. In studies under controlled conditions, the largest differences were noted after 12 hours of incubation. By 24 hours, most of the difference among cultivars had disappeared. This indicates that the differences in cultivar reaction may only be important in the field if the conditions are marginal for infection. Under prolonged periods of cool wet weather, these small differences may disappear. Cultivar reaction must be assessed in natural epidemics before recommendations on cultivar response can be made to growers.

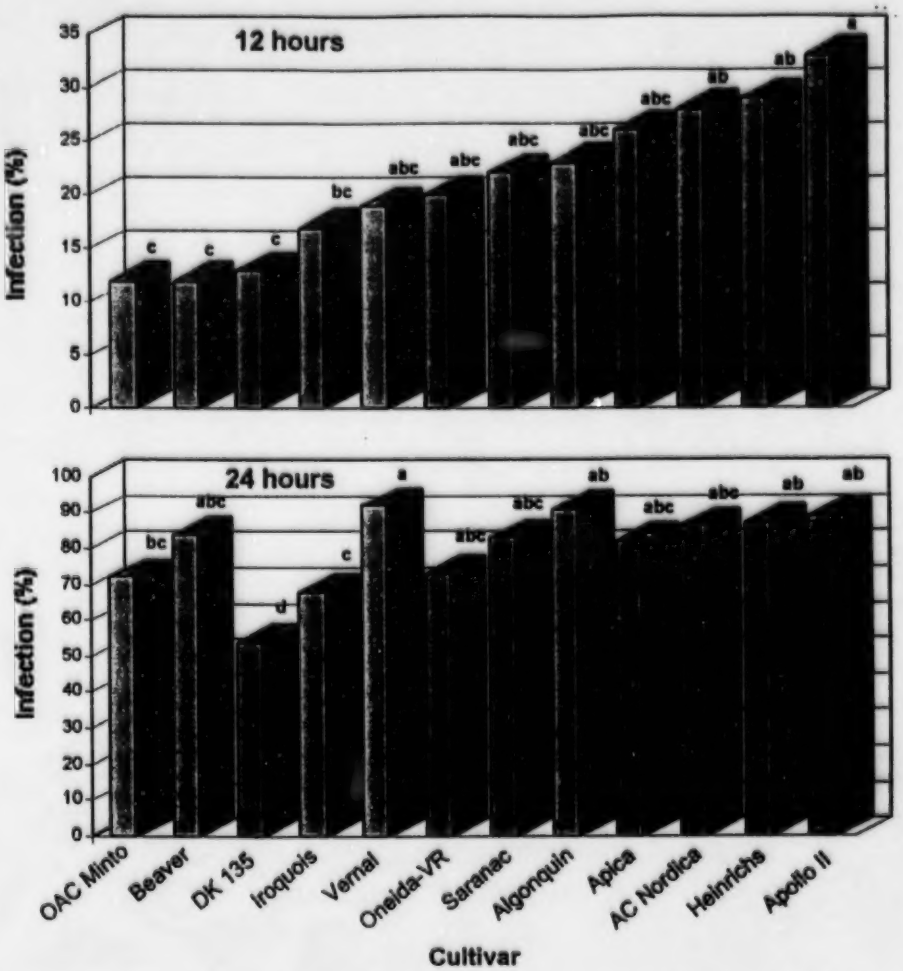

Figure 1. Incidence of flower infection by *Botrytis cinerea* (%) in inoculated, detached florets of 12 alfalfa cultivars at 12 and 24 hours after inoculation.

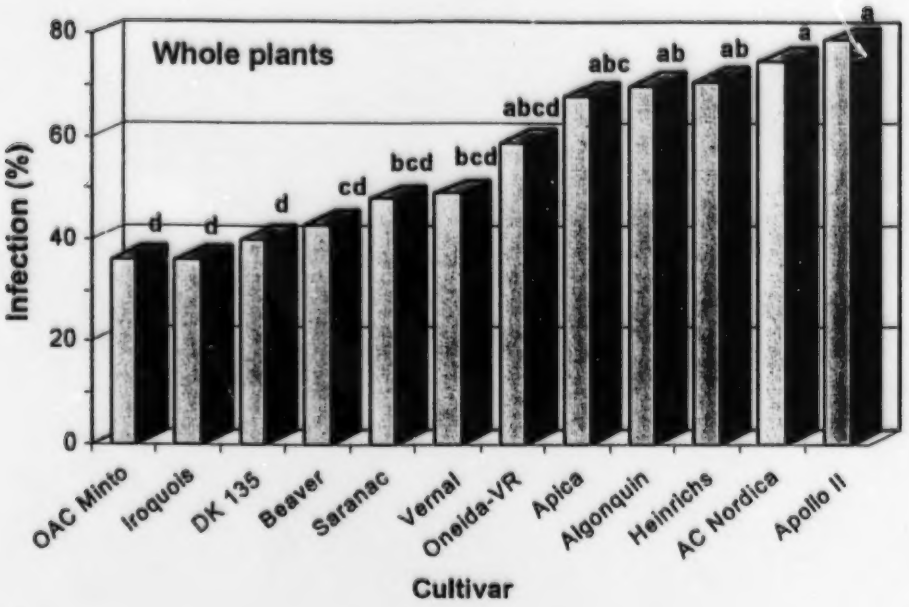

Figure 2. Incidence of flower infection by *Botrytis cinerea* (%) on inoculated plants of 12 alfalfa cultivars in greenhouse trials.

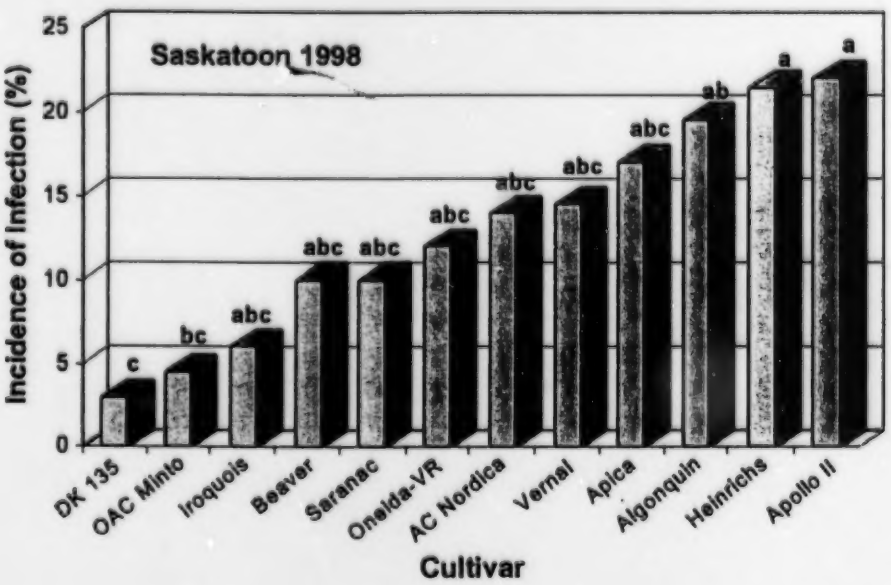

Figure 3. Incidence of flower infection by *Botrytis cinerea* (%) on 12 alfalfa cultivars, three days after inoculation, in field trials at Saskatoon in 1998 (n = 5).

The impact of flower orientation and color on infection by *B. cinerea* was also assessed under controlled conditions, to gain some understanding of the mechanisms that cause these differences among cultivars. The tests were conducted with inoculated, detached flowers that were incubated in small humidity chambers for 12 and 24 hours as described above. The experiments were replicated within each test, and the tests were repeated several times. The effect of flower colour was not consistent among cultivars (Fig. 4); purple flowers of cv. Iroquois had a lower incidence of infection than white flowers (61% vs. 81%), but there were no differences associated with flower color in Apollo II (purple vs. white) or AC Nordica (purple vs. yellow). Flowers of cv. Vernal that faced upward had a much lower incidence of infection (16% infected) than downward facing flowers (86%). This suggests that plant architecture may have a large impact on the disease reaction of cultivars; if so, we might be able to predict disease reaction of cultivars by assessing flower orientation in the absence of disease. This response also needs to be assessed in the field to evaluate its importance in disease resistance.

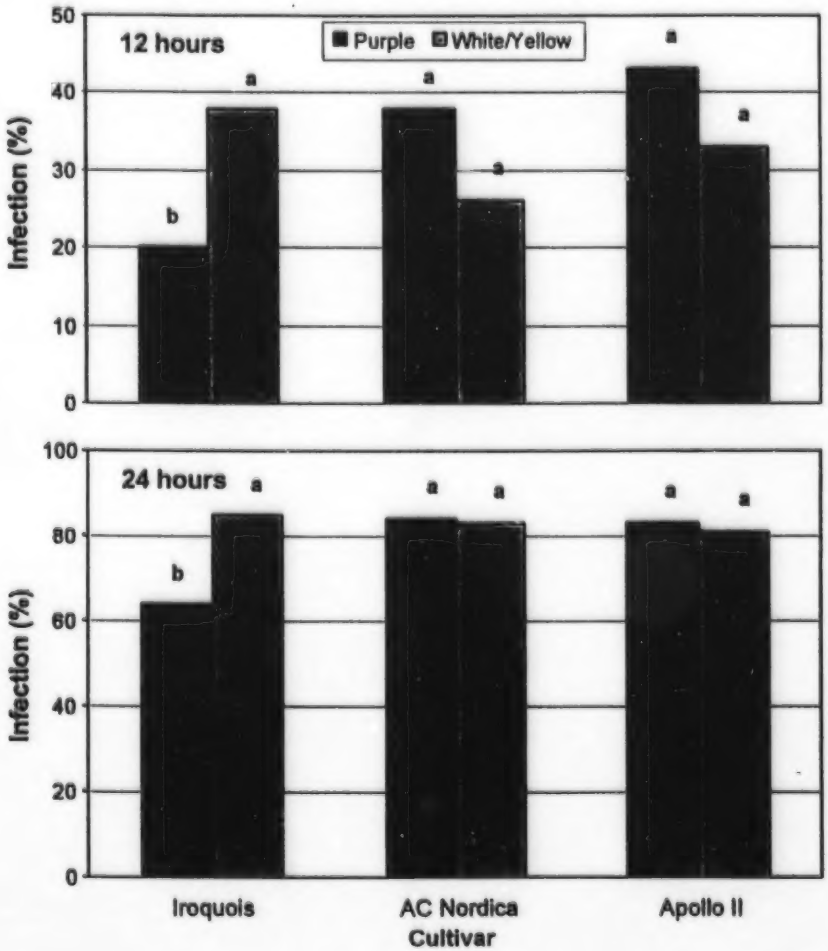

Figure 4. Impact of flower colour on the incidence of flower infection by *Botrytis cinerea* (%) on detached florets of three alfalfa cultivars 12 and 24 hours after inoculation.

Conclusion - Small but consistent differences in cultivar response to infection by *B. cinerea* were identified in studies under controlled conditions, but they need to be validated in the field before the information is promoted to growers. Differences in plant architecture among cultivars may have a large impact on infection.

Temperature and rainfall

1997-98 - The effects of air temperature and surface wetness duration on incidence of flower infection were examined in a replicated, factorial study that was repeated four times. Racemes of cv. Vernal were picked and inoculated with spores of *B. cinerea* (1×10^5 spores/ml). They were then placed in small humidity chambers and exposed to the temperature treatments (10, 15, 20, 25, or 30^{0}C). At regular intervals (0, 4, 8, 12, 16, 20, 24, 48 hr), 40 flowers per temperature were collected, surface-sterilized and plated onto PDA. The incidence of flower infection was rated 8-10 days later.

There was an interaction between temperature and wetness duration. The optimal temperature for infection was between 15-20^{0}C (Fig. 5). The pathogen required at least 12 hours of continuous wetness for all temperatures for infection. Incidence increased sharply after 12 hr at 20^{0}C. At temperatures below 15^{0}C or above 20^{0}C, longer periods of continuous wetness were required to produce high levels of flower infection. Very low levels of infection were observed at 30^{0}C, even after 48 hours of continuous wetness. This at least partially explains an observation from 1998, where heavy rainfall combined with very high temperatures did not affect blossom blight levels, although levels increased rapidly at locations that received the rain and moderate temperature.

Conclusion - The optimum conditions for infection of alfalfa flowers by *B. cinerea* were similar to those previously reported in the literature for several other host-pathogen systems. The main difference was that the period of flower wetness required to initiate infection was substantially longer than on other hosts. This information will be used to develop correlations between weather conditions and blossom blight outbreaks as part of a new project funded by AFIF.

Infection of Alfalfa Flowers by *B. cinerea*

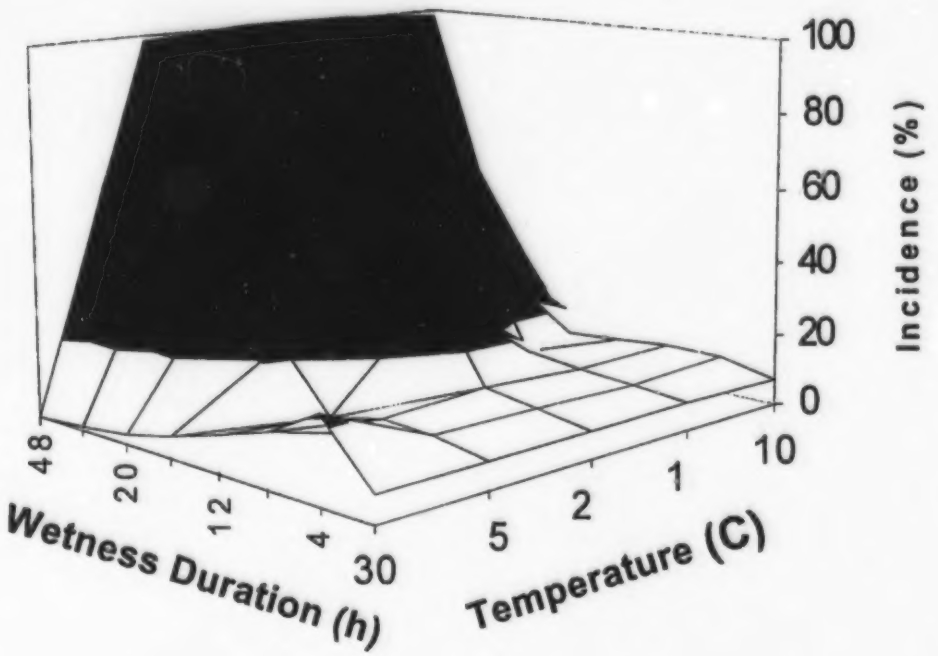

Figure 5. Impact of temperature and surface wetness duration on infection of detached alfalfa flowers by *Botrytis cinerea*.

Lentil

Disease survey

Objective: To determine the distribution and severity of botrytis blight of lentil in Saskatchewan.

Background - *Botrytis cinerea* had frequently been isolated at low levels from lentil seed in Saskatchewan, but had not been recognized as an important pathogen. However, in 1993 and 1994, it caused very substantial injury on lentil crops across the region.

1995 - Disease surveys were conducted in lentil fields in Saskatchewan in July and again in early to mid August. The crop was generally in a late vegetative stage in the first survey; lentil blossoms in 22 fields were sampled for *B. cinerea* in the second survey as described above for blossom blight of alfalfa. In southern and westcentral regions, levels of infection were generally less than 20% (Table 15). However, several fields with more than 50% blossom infestation were noted in the central region. This seems to be an important threshold, because all of the fields with more than 50% infestation also had visible stand loss due to botrytis stem and pod blight, while stands with lower levels did not have visible symptoms. Foliar disease severity was generally low, but anthracnose was severe at one site near Saskatoon and injury caused by *B. cinerea* and *S. sclerotiorum* was occasionally severe at sites near Regina and Saskatoon.

1996 - In 1996, 39 lentil fields in Saskatchewan were surveyed in July and again in August for disease. The lentil crops were generally not in flower in the first survey, so no blossom samples were taken. By early August, plants in some fields were setting seed, but lentil blossoms in 25 fields were sampled. At each field, 10 samples (5 flowers each, on aPDA) were taken along a teardrop-shaped transect of the field. The incidence of *S. sclerotiorum* was low (mean 3%, range 0 - 16%); *B. cinerea* occurred much more frequently (mean 22%, range 0 - 92%), with 9 fields having greater than 45% infection. Symptoms of botrytis blight were generally visible on leaves and stems in these fields. This supports the observation from 1995 that levels of flower infestation must be high before visible symptoms occur.

In the July survey, foliar disease levels were generally low. However, severe foliar blighting (generally caused by ascochyta blight, but also anthracnose and botrytis blight) was found in several fields by August, and all fields had at least a trace of foliar disease. Many of the fields that had moderate to severe foliar disease had been sprayed at least once with fungicide, so the producers were clearly aware of the developing disease situation and had taken corrective action.

1997 - The survey was not carried out because disease levels were extremely low. In 1998, a survey of anthracnose on lentil was carried out and reported as part of an AFIF project.

Conclusion - In 1993 and 1994, botrytis blight was very prevalent on lentil in Saskatchewan due to the cool, wet conditions that prevailed in most areas throughout the growing season. Wet conditions not only favour infection by *B. cinerea*, but also stimulate plant growth. The crop forms a dense canopy that often lodges and creates ideal conditions for disease development. In 1995, conditions were moderately favourable for botrytis blight and the survey identified regions

where the disease caused some injury. In 1996-98, there was little or no damage to lentil crops in Saskatchewan caused by *B. cinerea*. Clearly, this disease is extremely weather dependent and requires cool, wet conditions for epidemic development. We conclude that botrytis blight is less common on lentil than blossom blight on alfalfa, but that it is likely to reappear and cause losses in both yield and quality in wet years.

Table 15. The incidence of *Botrytis cinerea* and *Sclerotinia sclerotiorum* on lentil flowers in fields in Saskatchewan in 1995.

Location	% *B. cinerea*	% *S. sclerotiorum*
Central		
Saskatoon	95	0
Rosthern	58	8
Smuts	25	13
St. Denis	23	15
Aberdeen	78	40
Perdue	18	8
Delisle	20	15
Harris	13	3
Zealandia	33	3
Mean	**40**	**12**
Westcentral		
Unity	35	5
Rosetown 1	10	10
Rosetown 2	20	3
Rosetown 3	3	8
D'Arcy	48	13
Brock	5	8
Kindersley	8	0
Glidden	10	5
Wartime	20	10
Eston	13	18
Mean	**17**	**8**
Southcentral		
Bladworth	0	13
Girvin	10	3
Lumsden	45	0
Mean	**18**	**5**
Total = 23 fields		

Disease management using fungicides

Objective: To identify fungicides that provided cost-effective control of botrytis blight and are readily available to commercial alfalfa producers in western Canada.

1995 - A fungicide trial seeded to Eston lentil was established at Saskatoon. Seeding was delayed until early June in order to delay harvest into September, when the cool, wet conditions that favour infection and spread of *B. cinerea* are common. The same fungicides (Benlate, Bravo and Rovral at 1 and 2 applications vs. an untreated control) used in the alfalfa trials were included in this trial. Each plot was 3 x 2.5 m, with four replications in a randomized complete block design. The fungicides were applied on 15 and 25 August, when the plants were in full bloom and early pod set, respectively. The plot area was inoculated with infected plant material from a lentil field near Saskatoon in the interval between the first and second fungicide applications because there were few symptoms of infection with *B. cinerea* in the test. The plots were irrigated three times in September to increase the humidity and increase disease spread. Weather conditions at Saskatoon were not conducive for blight development throughout the early fall and very little stem and pod blight developed. The plots were harvested on 06 October.

No visible symptoms of grey mold were apparent in the plots, and there were no differences in yield (Table 16). Seed infection incidence was assessed by plating 50 seeds/plot onto aPDA. There was only 3% *B. cinerea*, 1% *S. sclerotiorum* and 1% *Ascochyta lentis* in the controls, so no further analysis was done.

Table 16. Effect of fungicides for *Botrytis cinerea* on lentil seed yield (kg/ha) at Saskatoon 1995.

Fungicide	Yield (kg/ha)
Control	690
Early Flower	
- Bravo	860
- Benlate	700
- Rovral	600
Early + Late	
- Bravo	800
- Benlate	770
- Rovral	650

There were no significant differences in yield in the trial.

1996 - Fungicide application was assessed in three growers' fields, which had been identified in the June survey as having botrytis blight symptoms or high levels of flower infestation. The fungicide treatments were as described previously for the alfalfa trials in 1996. In addition, two small plot fungicide trials were conducted on Laird lentil; one at the research farm at Saskatoon (mist irrigation) and the other at Melfort (dryland) in 1996.

Fungicides (Benlate, Bravo and Ronilan) were applied on 16 and 25 July to cv. Richlea at St.

Denis, 19 and 28 July to cv. Laird at Laura, and 22 July and 01 August to cv. Laird at Glenside. Blossom infestation was assessed by sampling (2 plates of 8 blossoms per plot) on 13 August at Laura and 15 August at Glenside. The plots were harvested (30 m^2 per plot) on 30 August at Laura, 13 September at St. Denis and 27 September at Glenside.

The results of the flower infestation and yield assessments are summarized in Table 17. Flower infestation levels exceeded the 50% threshold (from observations in 1995) only at St. Denis, but these fields represented the highest levels that were available in the region. There was no pattern of changes in flower infestation with fungicide treatment. At Glenside, levels of infestation in the control dropped to 0% prior to the late fungicide application date, due to hot, dry conditions that limited disease spread. There were low levels of grey mold symptoms in each field, but fungicide treatment did not affect severity. Yield differences were small and there was no clear association with treatment.

Seed quality characteristics were assessed at St. Denis and Glenside (Table 18). At Laura, no *B. cinerea* was detected in the controls, so no further analysis was carried out. Fungicide application reduced infection with *A. lentis*, but levels of *B. cinerea* were so low that no treatment effects were noted.

Table 17. Impact of fungicide applications on the incidence of *Botrytis cinerea (Bc)* in lentil flowers, disease severity (% leaf area affected) and seed yield (kg/ha) in three commercial lentil fields in Saskatoon in 1996.

Fungicide application	St. Denis		Laura			Glenside			
	Bc (%)	Yield (kg/ha)	*Bc* (%)	Severity (%)	Yield (kg/ha)	*Bc*1 (%)	*Bc* 2 (%)	Severity (%)	Yield (kg/ha)
Control	69	1880	31	8	1930	27	0	2	1790
Early Flower									
- Bravo	84	2320	18	4	2050	22	0	2	1810
- Benlate	78	1940	6	6	2040	19	6	4	1660
- Ronilan	53	1900	6	11	2110	18	13	2	1840
Late Flower									
- Bravo	84	2000	6	7	2020	-	0	5	1760
- Benlate	47	1980	13	12	1930	-	13	3	1730
- Ronilan	63	1880	25	5	1940	-	13	3	1820
Early + Late									
- Bravo	75	2040	6	3	2200	-	6	2	1850
- Benlate	81	1800	19	6	2020	-	0	3	1790
- Ronilan	78	1680	13	5	2140	-	0	4	1740

Table 18. Impact of fungicide applications on the incidence of *Botrytis cinerea (Bc)* and *Ascochyta lentis* (*Al*) in lentil seed from three fields in Saskatoon in 1996.

	St. Denis		Laura		Glenside	
Fungicide	*Al*	*Bc*	*Al*	*Bc*	*Al*	*Bc*
Control	24	1	14	0	40	0
Early Flower						
- Bravo	12	1	-	-	3	0
- Benlate	17	0	-	-	13	0
- Ronilan	31	3	-	-	23	1
Late Flower						
- Bravo	11	0	-	-	8	0
- Benlate	26	2	-	-	8	0
- Ronilan	30	0	-	-	7	0
Early + Late						
- Bravo	2	0	-	-	0	0
- Benlate	19	1	-	-	9	0
- Ronilan	23	1	-	-	11	0

A small-plot test at Saskatoon was inoculated to run-off with a spore suspension (10^6 spores/ml) of *B. cinerea* on 20 July and 02 August, then sprayed with fungicide on 02 and 12 August Blossom contamination was assessed on 09 and 19 August The plots were mist-irrigated for a short period (1 - 2 minutes) each evening from 20 July to 03 Sept. to provide conditions conducive for disease development. A similar trial was conducted at Melfort (rainfed site).

At Saskatoon, the incidence of blossom blight in the control treatment at the first sampling date was 36%. Early fungicide application reduced blossom infestation, but a later application had no effect on blossom infestation because levels were already very low (Table 19). Treatment did not affect seed yield and the incidence of *B. cinerea* in seed was essentially 0% in all treatments. Seed infection with *A. lentis* was high but there was no difference in incidence among treatments, although there was a pattern of lower incidence with fungicide treatment.

Conclusion - Foliar fungicide application occasionally reduced flower infection but did not affect yield in these trials. Two factors adversely affected the study. First, summer weather conditions in the final two years of the trial were very dry; disease did not develop in research plots that were irrigated daily and there was no disease in commercial fields. Second, the best application technology for late-season foliar fungicides needed to be identified, but weather conditions were never conducive for such studies. We observed in the disease surveys (described above) that botrytis blight was most prevalent in heavy lentil stands, probably because the dense canopy reduced air flow and retained humidity longer than open stands. Foliar fungicides applied late in the growing season with conventional sprayer equipment did not penetrate the canopy of these dense stands, and so had little potential for controlling a pathogen like *B. cinerea* that attacks leaves and stems. A range of new sprayers and nozzles are available, including a sprayer

with a high pressure air-assist that pushes the foliage aside to permit the spray to penetrate to the base of the canopy. However, we were unable to assess this new equipment because of weather constraints. Botrytis blight can develop quickly late in the growing season, so fungicide application before the canopy closes is also impractical.

Table 19. Impact of fungicide application on the incidence of *Botrytis cinerea* (*Bc*) on lentil blossoms, lentil seed yield (kg/ha) and seed infection by *Bc* and *Ascochyta lentis* (*Al*) in a small plot trial at Saskatoon in 1996.

Fungicide	Yield (kg/ha)	Flower infestation (% *Bc*)		Seed infection	
		09 Aug.	19 Aug.	% *Al*	% *Bc*
Control	1520ab	36a	0a	32a	1a
Early flower					
- Bravo	1860a	20b	13a	15a	0a
- Benlate	1700ab	13b	16a	15a	0a
- Ronilan	1360ab	14b	16a	24a	0a
Late flower					
- Bravo	1430ab	-	9a	19a	0a
- Benlate	1590ab	-	13a	20a	0a
- Ronilan	1490ab	-	13a	30a	0a
Early + Late					
- Bravo	1560ab	-	3a	26a	0a
- Benlate	1580ab	-	3a	22a	0a
- Ronilan	1280b	-	9a	20a	0a

Means within a column followed by the same letter are not significantly difference based on DMRT at $P \leq 0.05$.

Stand Density

Objective: To assess the impact of lentil stand density on botrytis blight development.

Background - In 1993-94, botrytis blight was severe in many lentil fields late in the season. Seed quality was so low in most of these field that most of it graded as Sample and was used as animal feed. Often, the stands that were uniform and complete suffered the most damage, while nearby fields where the stand was thin or spotty had less damage.

1995 - The plots were seeded at Saskatoon on 30 May in a split plot design, with cultivar (Eston vs. Laird) as the main plots and seeding rate (50, 75 and 100% of normal = 39 live seed per m row) as the subplots. Each subplot was 2.5 x 3.7m. The seed of Eston carried little or no disease, but there was a high level of *B. cinerea* in the Laird seed. Stand counts (# live plants and # dead plants in each of two 1m lengths of row per plot) were made on 30 June. Blossoms were plated from selected plots at irregular intervals throughout the flowering period, but the incidence of *B. cinerea* and *S. sclerotiorum* was very low (data not shown). The plots were mist-irrigated three times in September to stimulate disease development, but symptoms of botrytis blight did

not develop. The density of the stand (% ground cover) in each plot was assessed on 27 September and the plots were harvested on 04 October. Seed infection incidence was assessed by plating 50 seeds/plot onto aPDA. Seeding rate affected emergence, stand density and yield (Table 20), but botrytis blight did not develop in the plots, so the impact of density on disease could not be assessed. The high rate of seed infection in Laird was associated with differences in seed establishment and stand density, but did not have a measurable effect of the number of dead plants in the plots. Seed samples were assessed in agar plate tests; the mean incidence of *B. cinerea* and *A. lentis* was less than 1% (data not shown).

Table 20. Impact of seeding rate on establishment, stand density and seed yield (kg/ha) of lentil at Saskatoon in 1995.

Cultivar	Seeding rate (%)	# of live plants/m row	# of dead plants/m	% Cover (Sept. 27)	Yield kg/ha
Eston	100	16a	13a	81a	1930a
Eston	75	12b	10ab	79a	1770a
Eston	50	9c	4c	66bc	1310b
Laird	100	8c	11a	70b	820c
Laird	75	9c	8abc	74ab	860c
Laird	50	5d	6bc	61c	660c

100% seeding rate is 39 live seeds per m of row.
Means in a column followed by the same letter are not significantly different based on DMRT at $P \leq 0.05$.

1996 - The impact of stand density on botrytis blight of lentil was assessed at Saskatoon and Melfort. The design and treatments were as described above, except that the seed lots of both cultivars were free of *B. cinerea*. Stand establishment was assessed at Saskatoon on 08 July and the plots were irrigated every evening from 09 July until 15 september.

Botrytis blight did not develop at the Saskatoon site, but the irrigation did stimulate development of ascochyta blight late in the season. The 50% seeding rate had lower yields than the 100% rate (Table 21), but seed infection with *B. cinerea* was not affected.

At Melfort, a low level of botrytis blight developed late in the season (Table 22). Symptoms were most severe at the highest seeding rate in cv. Eston, but there was no effect of seeding rate on disease severity or yield. Seeding rate did not affect the incidence of *B. cinerea* in the seed, but incidence was higher in Laird than in Eston (based on analysis of variance).

1997 - Trials were conducted at Saskatoon and Melfort as described above. In addition to inoculation with a spore suspension, *B. cinerea* was grown on killed pea seed and distribute in the plots to ensure that high levels of inoculum were maintained. Little or no botrytis blight was noted at any date. Yields were not taken at Melfort. At Saskatoon, seeding rate had an impact on yield of Laird but not Eston (Table 23). Levels of *B. cinerea* in seed were quite low (7%) in the 100% Laird treatment, so no further analysis was conducted.

Table 21. Impact of seeding rate on yield of lentil at Saskatoon in 1996.

Cultivar	Seeding rate (%)	Yield kg/ha
Eston	100	1910b
Eston	75	1680c
Eston	50	1710bc
Laird	100	2280a
Laird	75	2180a
Laird	50	1800bc

100% seeding rate is 39 live seeds per m of row.
Means in a column followed by the same letter are not significantly different based on DMRT at $P \leq 0.05$.

Table 22. Impact of seeding rate on stand establishment, botrytis blight severity on foliage, seed yield and seed infection with *Botrytis cinerea* on lentil at Melfort in 1996.

Cultivar	Seeding rate (%)	# live plants/m row	Blight severity (%) 01 Aug	Blight severity (%) 29 Aug	Yield kg/ha	*B. cinerea* in seed (%)
Eston	100	14a	2a	12a	3380a	11ab
Eston	75	10b	1b	4b	3660a	11ab
Eston	50	10b	1b	5b	3270a	7b
Laird	100	9bc	1b	3b	2520b	25a
Laird	75	8bc	1b	2b	2150b	22a
Laird	50	6c	1b	3b	2290b	20ab

100% seeding rate is 39 live seeds per m of row.
Means followed by the same letter are not significantly different based on DMRT at $P \leq 0.05$.

Table 23. Impact of seeding rate on stand establishment and yield of lentil at Saskatoon in 1997.

Cultivar	Seeding rate (%)	# of live plants/m row	Yield kg/ha
Eston	100	22a	1850b
Eston	75	21a	2090ab
Eston	50	16b	1900b
Laird	100	16b	2480a
Laird	75	12bc	2230ab
Laird	50	10c	2040ab

100% seeding rate is 39 live seeds per m of row.
Means in a column followed by the same letter are not significantly different based on DMRT at $P \leq 0.05$.

1998 - Trials were conducted at Saskatoon and Melfort as described above. No botrytis blight was noted in the plots at Melfort at any date and yields were not taken. At Saskatoon, low levels of botrytis blight developed in a foliar epidemic where ascochyta blight and anthracnose were the dominant components, so a generalized blight assessment was made. Seeding rate did not affect establishment or have a consistent effect on yield in this trial (Table 24). Seed assessments on the Laird x 100% treatment revealed 8% infection with *B. cinerea* and 10% with *A. lentis*.

Table 24. Impact of seeding rate on stand establishment and yield of lentil at Saskatoon in 1998.

Cultivar	Seeding rate (%)	# of live plants/m row	Blight severity (%)	Yield kg/ha
Eston	100	14bc	22a	1830ab
Eston	75	15bc	16a	1810ab
Eston	50	12c	19a	2020a
Laird	100	22a	9b	1510bc
Laird	75	18ab	9b	1670abc
Laird	50	21a	11b	1320c

100% seeding rate is 39 live seeds per m of row.
Means in a column followed by the same letter are not significantly different based on DMRT at $P \leq 0.05$.
Blight severity was predominantly ascochyta blight, but anthracnose and botrytis blight were noted in some plots

Conclusion: Measurable levels of foliar infection with botrytis blight developed in only 1 of 7 station years for this trial. In that site (Melfort 1996), yields were very high, probably as a result of wet, cool conditions that promote disease development. Botrytis blight did not affect yields at that site, but levels of infection were higher in cv. Laird, which is taller, more prone to lodging, and later maturing than cv. Eston. This result provides some support for observations that botrytis blight is most severe in dense stands, but it is clear that reducing seeding density to avoid disease is not a prudent management option.

Cultivar reaction

Objective: To assess the disease reaction of lentil cultivars to botrytis blight under field conditions.

1996 - Cultivar reaction to *B. cinerea* was assessed on seven lentil cultivars (Laird, Eston, Indian Head, Rose, CDC Matador, CDC Gold, CDC Redwing), representing a range of genetic background and growth form, in small plot trials at Saskatoon and Melfort. At Saskatoon, each plot consisted of 4 rows, each 3 m long with 0.3 m inter-row spacing, arranged in a randomized complete block design with four replicates. At Melfort, each plots was 6 m in length. Both sites were inoculated with a spore suspension, plots at Saskatoon were mist-irrigated, and the trials were evaluated as described above.

At Saskatoon, botrytis blight was visible in the trial, but distribution was erratic and severity was low. As expected, there were substantial yield differences among the cultivars. As well, there

were significant differences in seed infection with *B. cinerea* and *A. lentis* among cultivars (Table 25). CDC Gold lacks tannin in its seed coat. The line was developed to reduce discoloration as the seed ages, which is caused by changes in the tannins in the seed coat. However, these data confirm previous observations that this cultivar is very susceptible to *A. lentis*. It also demonstrates that the line is more susceptible to *B. cinerea* than the other lines in the trial. Indian Head, CDC Matador and CDC Redwing are all partially resistant to *A. lentis*.

As has been discussed before, weather conditions at Melfort in 1996 were conducive for development of botrytis blight late in the season. In early August, levels of *B. cinerea* on foliage and flowers were low, but levels increased rapidly close to harvest. There were differences among cultivars in botrytis blight severity on leaves and flowers, but the pattern of differences varied substantially between dates. Levels of seed infection were high, with CDC Matdor, Indian Head and Eston having lower levels of infection than the other cultivars.

Table 25. Cultivar reaction to *Botrytis cinerea* in an inoculated, irrigated test at Saskatoon, 1996.

Cultivar	Yield kg/ha	% seed infection with	
		B. cinerea	*A. lentis*
Laird	1320cd	3b	32c
Eston	2550ab	3b	40bc
Indian Head	2970a	1b	6d
Rose	1990bc	4b	43b
CDC Matador	3010a	2b	10d
CDC Redwing	1940bc	4b	4d
CDC Gold	860d	11a	70a

Means in a column followed by the same letter are not significantly different based on DMRT at $P \leq 0.05$.

Table 26. Cultivar reaction to *Botrytis cinerea* in a test at Melfort, 1996.

Cultivar	Foliar blight severity (%)			% in flowers		Yield	*B. cinerea*
	Aug01	Aug15	Aug29	Aug01	Aug14	kg/ha	in seed (%)
Laird	2a	23ab	20bcd	23a	80ab	1960cd	75a
Eston	1b	13bc	29abc	24a	89a	3470a	36cd
Indian Head	1b	4c	11cd	10ab	68b	2400bc	30cd
Rose	1b	11bc	14bcd	8b	75ab	2900b	48bc
CDC Matador	1b	31a	33ab	6b	86ab	2750b	21d
CDC Redwing	1b	39a	41a	16ab	81ab	2690b	72a
CDC Gold	1b	5c	8d	16ab	68b	1440d	60ab

Means in a column followed by the same letter are not significantly different based on DMRT at $P \leq 0.05$.

1997 - The trial was conducted at Saskatoon and Melfort, with a layout and treatments as described above. In addition to inoculation with a spore suspension, *B. cinerea* was grown on

killed pea seed and distributed in the plots to ensure that levels of inoculum were maintained at high levels throughout July. At Saskatoon, mist irrigation was applied for 3 min at 9:00 pm each day from 03 July to 10 September to increase the humidity in the plots without unduly affecting water relations in the plants. The spore and grain inoculum was applied on 01 August. Visual assessments (HB ratings) of botrytis infection of leaves, stems, flowers/pods were made at about 10 day intervals starting 10 days after inoculation. No botrytis blight was noted on any date at either site. No seed harvest was made at Melfort. Stand establishment and yield means for Saskatoon are presented in Table 27. No *B. cinerea* was detected in seed samples in preliminary tests, so seed plating to assess infection incidence was halted.

Table 27. Cultivar reaction to *Botrytis cinerea* in an inoculated, irrigated test at Saskatoon, 1997.

Cultivar	# of live plants/m row	Yield kg/ha
Laird	17ab	2152ab
Eston	22a	2561a
Indian Head	16ab	2664a
Rose	18ab	1733bc
CDC Matador	26a	2313ab
CDC Redwing	24a	1970b
CDC Gold	8b	1210c

Means in a column followed by the same letter are not significantly different based on DMRT at $P \leq 0.05$.

1998 - The trial was conducted at Saskatoon and Melfort. Design, inoculation and assessments were as described for 1997. No disease developed at Melfort, and so no seed harvest was taken. At Saskatoon, a severe anthracnose epidemic occurred, with trace levels of *B. cinerea* and *A. lentis*. The ascochyta-resistant cv. Redwing is extremely susceptible to anthracnose, which explains its low yield in this trial (Table 28). Seed assessments on Laird seed revealed 8% *B. cinerea* and 10% *A. lentis*. Assessments of the remaining seed will be completed as time permits.

Table 28. Cultivar reaction to *Botrytis cinerea* in an inoculated, irrigated test at Saskatoon, 1998.

Cultivar	# of live plants/m row	Foliar severity (%), 06 Aug	Yield kg/ha
Laird	23a	48ab	1145b
Eston	21a	41abc	1191b
Indian Head	20a	9c	1806a
Rose	21a	26bc	1996a
CDC Matador	23a	33abc	1781a
CDC Redwing	23a	63a	627c
CDC Gold	15a	31bc	631c

Means in a column followed by the same letter are not significantly different based on DMRT at $P \leq 0.05$.

Conclusion - CDC Gold consistently had the highest levels of seed infection with *B. cinerea* and *A. lentis*, and also had the lowest establishment in the trial, probably because of its extreme susceptibility to damping off pathogens. Differences among the other cultivars were not consistent between rating dates and sites. Blight development was only severe in 1 of 7 station years. At that site, seed infection was substantially higher on CDC Gold, Laird and CDC Redwing than on Eston.

Growth Stage

Objective: To determine if pulses are differentially susceptible to botrytis blight at different growth stages.

Background - Botrytis blight often occurs on lentil seedlings, which then develop stem infections near the soil surface and die. The pathogen then spreads to adjacent seedlings within the row, creating gaps in the stand that are rapidly filled with weeds. After this initial period of infection, symptoms are rarely seen until after the plant canopy has closed. Stem, flower and pod injury can be very severe under cool, wet conditions. Young leaves may be resistant to infection, or infection may be present but only express symptoms as the tissues start to senesce. More likely, young plants avoid infection because the canopy is not dense enough to create a moist micro-environment; the pathogen could survive on plant residue until conditions for infection improve. Infection on various plant parts has been studied on several other hosts, and differences in susceptibility among plant organs of different ages have been identified in some, but not all, of these systems. In chickpea, genes for partial resistance to *B. cinerea* have been reported in the literature, but are not available in adapted genotypes.

1997 - Laird lentil, Carneval field pea, and AC-27 kabuli chickpea were planted at high seeding density in field plots at Saskatoon on 4 dates, at about 10 day intervals starting in mid May, to provide a range of growth stages. Each plot was 3 x 2 m, with 0.3m inter-row spacing in an RCBD with 4 replicates. Production of inoculum, inoculation dates, irrigation and assessment of disease were as described above. No botrytis blight was noted at any date in this trial. Levels of *B. cinerea* in the infected seed were extremely low (not shown). The trial was not repeated.

1998 - Chickpea seed of cv. Arizonia (desi) and cv. Sanford (kabuli) was surface disinfested and planted in soilless mix on three dates at 2-week intervals to provide a range of ages of plant material for the study. Two isolates of *B. cinerea* (De9702 from lentil and Jan9703 from chickpea) were used as inoculum sources. Spore suspensions (1 x 10^5 spores/ml) of each isolate were prepared as described previously. The test was arranged in a split plot design, with cultivar (Arizonia vs Sanford) as the main plots. The subplots treatments were a factorial arrangement of the following three factos; 1) tissue age (young vs old), 2) tissue type (leaves, flowers and pods)and 3) isolate (De9702, Ja9703 or sterile water). Young leaves were chosen from the top 2 - 3 nodes and old leaves were chosen from the bottom nodes of the plants. Young flowers were closed and showing colour or just beginning to open, and old flowers were fully expanded. Young pods were small and flaccid, with small, undeveloped seeds; old pods were large, full, and green to brown in colour.

The plant samples were collected and inoculated to run-off with one of the *B. cinerea* isolates using an atomizer. They were then placed in small humidity chambers with 3 samples of each treatment (e.g. 3 young leaves of Arizonia, inoculated with De9702) per chamber, 2 chambers per experimental unit and 2 replicates per test. Two chambers of each tissue x cultivar combination were included as controls. The chambers were incubated at approximately 20^0C and rated (0-3 scale) for symptoms of grey mold after 4-5 days. The identity of the pathogen was confirmed by isolation. The experiment was repeated. Leaf, flower, and pod means were analyzed separately using analysis of variance.

Infection severity was higher on flowers (mean of 2.6) than leaves on (2.0) and pods (1.7). There were no differences between the cultivars on leaves or flowers. On pods, severity was higher ($P<0.001$) on desi pods (2.1) than on kabuli pods (1.4). Flower age did not influence disease severity, but young leaves developed less disease (1.7) than old leaves (2.2) ($P<0.05$). In contrast, young pods (1.9) were more susceptible than old pods (1.6). Observations outside of the treatment structure showed that very young pods were more susceptible than slightly larger, fuller pods. Similarly, pods that had begun to senesce were more susceptible than green, mature pods. There were no differences between the isolates of *B. cinerea*. This work was done by Mr. R. Beaujot as part of an undergraduate thesis in Agriculture at the University of Saskatchewan.

In preliminary trials on lentil, a similar pattern of injury was observed, with the most severe and consistent infection on flowers and very young pods. Leaf age had little or no effect on susceptibility of lentil.

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Personnel - A list of the personnel employed on this project has already been submitted from the University of Saskatchewan. The only long-term employment from the grant was for an M.Sc. student in the Department of Biology at the U of S, Mr. Z. Lan. The grant supported his work for the whole three year term of his M.Sc. degree. A summer student was hired each year to support the field parts of the program and short-term technicians were hired to complete seed cleaning and seed quality evaluations.

Equipment - None purchased using these funds.

Project Developed Materials - Copies of the papers and publications from this study are available from the author upon request.

Project Photos - None.

Acknowledgement - All of the papers and posters on material from this work included a written acknowledgement of each of the groups that provided funding. This was also highlighted on the title slides in all presentations on this work.

Expense statement - Previously submitted by the University of Saskatchewan.